

WHAT IS CLAIMED IS:

1. Synthetic peptides comprising the regulatory virus protein R (Vpr) of the human immunodeficiency virus type 1(HIV-1).
- 5 2. The method of Claim 1, wherein said that peptides are characterized as follows
- 2.1. as a 96 amino acid long Vpr protein ($sVpr^{1-96}$)
- 2.2. as a 47 amino acid long N-terminal peptide ($sVpr^{1-47}$)
- 2.3. as a 49 amino acid long C-terminal peptide ($sVpr^{48-96}$), as well as
- 2.4. fragments of those peptides thereof, for example
- 10 2.4.1. overlapping, approximately 15 amino acid long peptides for epitope-characterization and isoelectric focusing
- 2.4.2. approximately 20 amino acid long peptides for structural and functional characterization of individual domains of Vpr, especially
- 2.4.2.1. the peptide $sVpr^{1-20}$ and
- 15 2.4.2.2. the peptide $sVpr^{21-40}$
3. Peptides of claims 1 and 2, wherein said that peptides are characterized as follows
- 3.1. the 96 amino acid full lenght Vpr-Protein $sVpr^{1-96}$ comprises the sequence:
- 20 H - Met - Glu - Gln - Ala - Pro - Glu - Asp - Gln - Gly - Pro - Gln - Arg - Glu - Pro - Tyr - Asn - Glu - Trp - Thr - Leu - Glu - Leu - Leu - Glu - Glu - Leu - Lys - Ser - Glu - Ala - Val - Arg - His - Phe - Pro - Arg - Ile - Trp - Leu - His - Asn - Leu - Gly - Gln - His - Ile - Tyr - Glu - Thr - Tyr - Gly - Asp - Thr - Trp - Ala - Gly - Val - Glu - Ala - Ile - Ile - Arg - Ile - Leu - Gln - Gln - Leu - Leu - Phe - Ile - His - Phe - Arg - Ile - Gly - Cys - Arg - His - Ser - Arg - Ile - Gly - Val - Thr - Arg - Gln - Arg - Arg - Ala - Arg
- 25 - Asn - Gly - Ala - Ser - Arg - Ser - OH
- 3.2. the 47 amino acid long N-terminal Peptid $sVpr^{1-47}$
- H - Met - Glu - Gln - Ala - Pro - Glu - Asp - Gln - Gly - Pro - Gln - Arg - Glu - Pro - Tyr - Asn - Glu - Trp - Thr - Leu - Glu - Leu - Leu - Glu - Glu - Leu - Lys - Ser - Glu

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- Ala - Val - Arg - His - Phe - Pro - Arg - Ile - Trp - Leu - His - Asn - Leu - Gly - Gln
- His - Ile - Tyr - NH₂

3.3. the 49 amino acid long C-terminal peptide *sVpr*⁴⁸⁻⁹⁶

5 Glu - Thr - Tyr - Gly - Asp - Thr - Trp - Ala - Gly - Val - Glu - Ala - Ile - Ile - Arg -
Ile - Leu - Gln - Gln - Leu - Leu - Phe - Ile - His - Phe - Arg - Ile - Gly - Cys - Arg -
His - Ser - Arg - Ile - Gly - Val - Thr - Arg - Gln - Arg - Arg - Ala - Arg - Asn - Gly -
Ala - Ser - Arg - Ser-OH

10 3.4. fragments of those peptides thereof consisting of 15 amino acid long peptides

3.4.1. *sVpr*¹¹⁻²⁵

Gln - Arg - Glu - Pro - Tyr - Asn - Glu - Trp - Thr - Leu - Glu - Leu - Leu - Glu - Glu

15 3.4.2. *sVpr*⁴¹⁻⁵⁵

Asn - Leu - Gly - Gln - His - Ile - Tyr - Glu - Thr - Tyr - Gly - Asp - Thr - Trp - Ala

3.4.3. *sVpr*⁴⁶⁻⁶⁰

Ile - Tyr - Glu - Thr - Tyr - Gly - Asp - Thr - Trp - Ala - Gly - Val - Glu - Ala - Ile

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3.4.4. *sVpr*⁵⁶⁻⁷⁰

Gly- al-Glu-Ala-Ile-Ile-Arg-Ile-Leu-Gln-Gln-Leu-Leu-Phe-Ile

3.5. as the approximately 20 amino acid long peptides

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3.5.1. the peptide *sVpr*¹⁻²⁰ in form of

*sVpr*¹⁻²⁰(Asn^{5,10,14})

H-Met - Glu - Gln - Ala - Asn - Glu - Asp - Gln - Gly - Asn - Gln - Arg - Glu - Asn -
Tyr - Asn - Glu - Trp - Thr - Leu-NH₂, and

3.5.2. the peptide $sVpr^{21-40}$ in form of
 $sVpr^{21-40}(Asn^{35})$

H-Glu - Leu - Leu - Glu - Glu - Leu - Lys - Ser - Glu - Ala - Val - Arg - His - Phe -
5 Asn - Arg - Ile - Trp - Leu - His-NH₂

4. Method for the production of synthetic peptides derived from the regulatory virus
proteins R (Vpr) of the human immunodeficiency virus type 1 (HIV-1) according to
claims 1 to 3, wherein said that the synthesis of C-terminal Vpr-peptides is performed
10 on a serine resin using a Perkin Elmer synthesizers and all N-terminal peptides are
synthesized on a polystyrene polyoxyethylen resin. All peptides were synthesized
using Fmoc protection group strategy.

5. Method of claim 4, wherein said that at the end of the synthesis protection groups
15 are cleaved off using a cleavage mixture consisting of 95% trifluoroacetic acid, 3%
triisopropylsilane, and depending on the peptide sequence 2 to 5 % ethandithiol, and
the resin was separated.

6. Methods of claims 4 and 5, wherein said that the raw peptides were purified by
20 HPLC chromatography on a column of silica gel using a linear gradient of TFA and
water in acetonitrile.

7. Application of synthetic peptides derived from the regulatory virus proteins R
(Vpr) of the human immunodeficiency virus type 1 (HIV-1) for therapeutic and
25 diagnostic use.

8. Application of claim 7

8.1. in biological assays

8.1.1. for the development of serological test systems

- 8.1.2. for the development of Vpr antigen capture ELISA (enzyme linked immune sorbent assays)
- 8.2. for the generation of antibodies directed against HIV peptide sequences
- 8.3. for the generation of anti-viral reagents
- 5 8.4. for the development of test systems to screen potential Vpr inhibitors.
- 8.5. for the development of cell culture and animal models to investigate pathological mechanism of Vpr
- 8.6. for structural analyses of Vpr and its domains
- 8.7. for application in *in vitro* assembly of viruses and the development of vectors
- 10 for application in gene therapy.
9. Applications according to claims 7 and 8, wherein said that those proteins are derived from sVpr in which the N-terminal domain is mutated in either one, some, or all of the four proline residues.
- 15 10. Application according to claims 7 to 9 for the production of poly- and monoclonal antibodies or antisera specific for Vpr.
11. Application claims 7 to 10 for the production of epitope-different Vpr specific
- 20 antibodies.
12. Application according to claims 7 to 11 in serological test systems.
13. Application according to claims 7 to 12 in a Vpr antigen (Ag) ELISA.
- 25 14. Application according to claims 7 to 13 as standard antigen for the calibration of Vpr-Ag-ELISA techniques.

15. Application according to claims 7 to 8 for the detection and for the estimation of Vpr concentration of viral Vpr in peripheral blood of HIV infected individuals.
16. Application of sVpr proteins according to claims 7 and 8 for *in vitro* test systems
5 for the characterization of Vpr Inhibitors.
17. Application according to claims 7 and 8 for complementation of the function of endogenous viral Vpr in cell cultures infected with *vpr* deficient HIV mutants.
- 10 18. Application according to claims 7, 8 and 17 for complementation of the function of endogenous viral Vpr in cultures of primary human lymphocytes infected with *vpr* deficient HIV mutants.
- 15 19. Application according to claims 7, 8, 17 18 zur for complementation of the function of endogenous viral Vpr in cultures of primary human monocytes / macrophages infected with *vpr* deficient HIV mutants.
20. Application according to claims 7 to 19 for the characterization of reagents which
a) block the interaction of Vpr with cellular factors, like the glucocorticoid-receptor,
20 transcription factors and other DNA interacting enzymes and factors;
b) block the transcription activating activity of Vpr, and regulate, interfere with or block the activity of Vpr on steroid hormones;
c) regulate, interfere with , or block the transport of Vpr on its own or in complex with other components of the HIV preintegration complex as well as regulate,
25 interfere with, or block the encapsidation of Vpr in budding virus particles during the HIV assembly;
d) regulate, interfere with, or block the Vpr induced cell cycle arrest as well as regulate, interfere with, or block the effect of Vpr on cell differentiation and cell growth;

- e) regulate, interfere with, or block the cytotoxic effect of Vpr;
- f) regulate, interfere with, or block the ion channel activity of.

21. Application of sVpr proteins according to claims 7 and 8 for *in vivo* test systems
5 that characterize Vpr inhibitors.

22. Application of sVpr proteins according to claims 7 and 8 in animal model studies
for the characterization of functions according to claim 20.

10 23. Application of sVpr proteins according to claims 7 and 8 for the production of
concentrated peptide solutions.

24. Application of sVpr proteins according to claims 7, 8 and 23 for the production of
Vpr specific inhibitors.
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25. Application of sVpr proteins according to claims 7, 8, 21 and 24 for the
application of structure stabilizing factors that reduce the flexibility of the N-terminal
domain in Vpr.

20 26. Application according to claim 25, wherein said that those structure stabilizing
factors are

- a) the UBA2 domain of the DNA repairing enzyme HHR23A which bind to Vpr,
- b) Fab fragments of Vpr specific immune globulins,
- c) viral factors, especially components of the HIV-1 Gag polyprotein precursor
- 25 Pr55^{Gag} which interacts with Vpr during the process of Virus assembly; or
- d) the human glucocorticoid receptor or components thereof.

27. Application of sVpr proteins according to claim 7 for *in vitro* assembly of
retroviral preintegration complexes.

28. Application of sVpr proteins according to claims 7, 8 and 27 in *in vitro* or *in vivo* application of gene transfer methods.

5 29. Application of sVpr proteins according to claims 7, 8 and 28 for transfection, integration into chromosomal and episomal host DNA or any other gene transfer methods in eukaryotic cells.

10 30. Application of sVpr proteins according to claims 7, 8 and 28 for gene transfer using *in vitro* synthesized and / or manipulated genes and fragments thereof for the transfer into cells, tissues, and organisms, and any application for the purpose of gene therapy.

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